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| EXAMINER | |
|---|---|
| SCHLAPKOHL, WALTER | |

| ART UNIT | PAPER NUMBER |
|---|---|
| 1636 | |

DATE MAILED: 10/06/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/789,186 | MURPHY, KENAN C. | |
| | Examiner | Art Unit | |
| | Walter Schlapkohl | 1636 | waf |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *10 July 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1-43* is/are pending in the application.

4a) Of the above claim(s) *27-43* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1-26* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☒ The drawing(s) filed on *26 February 2004* is/are: a)☒ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *3/1/2006*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

**DETAILED ACTION**

Receipt is acknowledged of the papers filed 3/1/2006 and 7/10/2006 in which claims 1-2, 4-5 and 7-14 were amended. Claims 1-43 are pending in the instant application. Claims 27-43 are withdrawn. Claims 1-26 are under examination in the instant Office action.

***Claim Rejections - 35 USC § 112***

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1, 3-4, 6 & 8-13, and therefore dependent claims 2, 5, 7 & 14-26 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. **These are new rejections necessitated by Applicant's amendment.**

Claim 1 recites "[a]n isolated nucleic acid molecule comprising: (a) two nucleotide sequences encoding a bacteriophage recombinase; (b) a nucleotide sequence encoding a bacteriophage anti-recombinase…" in lines 1-5 (emphasis added).

Claim 1 is vague and indefinite in that it is unclear whether Applicant intends an anti-recombinase which is wild-type to a bacteriophage or whether Applicant intends an anti-recombinase which, e.g., inhibits the bacteriophage recombinase recited in component (a) of the claim, or both.

Similarly, claim 9 recites "a nucleotide sequence encoding a bacteriophage anti-recombinase" in lines 4-5. Claim 9 is vague and indefinite in that it is unclear whether Applicant intends an anti-recombinase which is wild-type to a bacteriophage or whether Applicant intends an anti-recombinase which, e.g., inhibits the bacteriophage recombinase recited in component (a) of the claim, or both.

Claim 3 recites the limitation "the origin of replication" in lines 1-2. There is insufficient antecedent basis for this limitation in the claim because there can be more than one origin of replication in the claim from which it depends.

Similarly, claims 6, 10 and 12 recite "the origin of replication" and the claims are vague and indefinite because the recitation of such a limitation has insufficient antecedent basis. Does Applicant intend a P22 anti-RecBCD or a λ gam sequence or both?

Claim 4 recites "[a]n isolated nucleic acid molecule comprising: (a) two nucleotide sequences encoding bacteriophage

λ Red recombinase; (b) a nucleotide sequence encoding bacteriophage λ anti-RecBCD…" in lines 1-4 (emphasis added). Claim 4 is vague and indefinite in that it is unclear what is meant by a bacteriophage λ anti-RecBCD; does Applicant intend a bacteriophage P22 anti-RecBCD (as found in the literature, e.g., Murphy, K.C., *J. Biol. Chem.* **269**(36):22507-22516, 1994, see entire document especially paragraph bridging pages 22507-22508), or does Applicant intend a bacteriophage λ gam sequence?

Similarly claims 8, 11, and 14 recite nucleotide sequences encoding "λ anti-RecBCD"

Claim 13 recites the limitation "the nucleotide sequence encoding-bacteriophage λ Red recombinase" in lines 1-2. There is insufficient antecedent basis for this limitation in the claim because there is more than one sequence which encodes the bacteriophage λ Red recombinase in the claim from which claim 13 depends.

***Claim Rejections - 35 USC § 112***

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1-26 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a new rejection not necessitated by Applicant's amendment.**

The claims are drawn to isolated nucleic acid sequences comprising (a) nucleotide sequences encoding a bacteriophage recombinase (b) nucleotide sequences encoding a bacteriophage anti-recombinase, $P_{tac}$ promoter sequences and nucleotide sequences encoding LacI operably linked to its native promoter. The claims encompass any nucleic acid comprising any sequence as long as the nucleic acid comprises a sequence which encodes a bacteriophage recombinase, a bacteriophage anti-recombinase and any $P_{tac}$ promoter sequence and lacI. The claims do not provide any structural information with regard to the recombinase or anti-recombinase sequences which are operably linked to a promoter. Nor do the claims provide any structural information with regard to the $P_{tac}$ promoter sequences capable of being operably linked to the nucleotide sequences of components (a)

and (b). Thus, the rejected claims comprise a set of nucleic acid sequences that are defined by their function.

To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of a complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, and any combination thereof. The specification describes nucleic acids for engineering bacterial chromosomes and teaches that the λ Red system, consisting of bet (a ssDNA annealing protein), exo (a 5'-3' dsDNA exonuclease) along with λ gam (an anti-RecBCD functioning protein) promote gene replacement into pathogenic bacteria such as *Escherichia coli* K-12 with high efficiency (see entire document, especially page 6, lines 6-24). The specification defines "recombinase" as "an enzyme, enzymatic activity of enzymatic function that catalyzes recombination" and teaches bacteriophage λ Red as encoded by exo and bet nucleotide sequences as a particularly preferred embodiment (page 10, lines 8-12). The specification also defines "anti-recombinase" as an "inhibitor of a recombinase activity endogenous to the host organism" and teaches the bacteriophage anti-recombinase gam as a preferred embodiment

(page 10, lines 13-16). The specification also teaches a nucleic acid sequences for the λ Red and gam coding regions (see SEQ ID NOs: 35-37). The specification describes a $P_{tac}$-red-gam operon used for the expression of λ exo, bet and gam sequences (see e.g., page 52, lines 17-18). The $P_{tac}$ promoter is also described as present in a the pKM200 and pKM201 vectors (see, e.g., page 27). However, no description is provided of a single $P_{tac}$ promoter sequence. No description is provided of a recombinase other than λ Red. No description is provided of an anti-recombinase other than λ gam. No description is provided of any other nucleic acid sequences comprising a recombinase, an antirecombinase, a $P_{tac}$ promoter and a lacI gene under the control of its native promoter other than a $P_{tac}$-exo-bet-gam-cI operon which is capable of inducing efficient recombination in pathogenic bacteria.

Even if one accepts that the examples described in the specification meet the claim limitations of the rejected claims with regard to structure and function, the examples are only representative of one nucleic acid sequence for each of the claimed nucleic acids components. The results are not necessarily predictive of any other recombinase sequences which can be used in conjunction with any other anti-recombinase sequences which can further be used in conjunction with any

other $P_{tac}$ promoter sequences such that 1) they are operably linked and 2) the nucleic acid is capable of inducing efficient recombination between two or more nucleic acids. Thus, it is impossible to extrapolate from the example(s) described herein those nucleic acid molecules that would necessarily meet the structural/functional characteristics of the rejected claims.

The prior art does not appear to offset the deficiencies of the instant specification in that it does not describe a set of bacteriophage recombinase/anti-recombinase/$P_{tac}$ promoter sequences other than λ Red and gam which can be used to promote efficient recombination, especially in pathogenic microorganisms such as enteropathogenic and enterohemorrhagic bacteria. Datsenko et al (*PNAS* **97**(12):6640-6645, 2000; of record) describe nucleic acids comprising λ Red and gam capable of inactivation of chromosal genes in E. coli K-12, but Datsenko et al do not teach that such would be possible with other recombinases or anti-recombinases. Furthermore, the literature shows that one of ordinary skill in the art would have quite a number of bacteriophage recombinases from which to choose. For example, Groth et al (*J. Mol. Biol.* **335**:667-678, 2004) teach any number of phage recombinase family members, such as tyrosine and serine integrases as well as tyrosine and serine recombinases from

phage λ, HK022, P22, etc. (see entire document, especially page 669, Table 1).

Given the very large genus of nucleic acid molecules encompassed by the rejected claims, and given the limited description provided by the prior art and specification with regard to the nucleic acid sequences (other than those disclosed) capable of efficient recombination of DNA, especially within pathogenic bacteria, the skilled artisan would not have been able to describe the broadly claimed genus of nucleic acid sequences comprising a recombinase, an anti-recombinase, a $P_{tac}$ promoter sequence and a lacI gene such that they would together promote efficient recombination within a bacteria. Thus, there is no structural/functional basis provided by the prior art or instant specification for one of skill in the art to envision those nucleic acid sequences that satisfy the functional limitations of the claims. Therefore, the skilled artisan would have reasonably concluded Applicant was not in possession of the claimed invention for claims 1-26.

***Claim Rejections - 35 USC § 102***

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless -
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

Claims 1, 4 and 7-8 are rejected under 35 U.S.C. 102(b) as being anticipated by Poteete et al (*Journal of Bacteriology* **181**(17):5402-5408, 1999). **This is a new rejection not necessitated by Applicant's amendment.**

Poteete et al teach a vector comprising: (a) two nucleotide sequences encoding a bacteriophage recombinase (λ exo and bet); (b) a nucleotide sequence encoding a bacteriophage anti-recombinase (λ gam); a $P_{tac}$ promoter sequence operably linked to the nucleotide sequences of (a) and (b): and a nucleotide sequence encoding LacI operably linked to its native promoter (see, e.g., description of pTP822 and pTP810 on page 5403, second column, 2nd full paragraph).

***Claim Rejections - 35 USC § 103***

The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

Claims 1-22 are rejected under 35 U.S.C. 103(a) as being unpatentable over Datsenko et al (*PNAS* **97**(12):6640-6645, 2000; of record) in view of Stewart et al (US Patent No. 6,355,412; of

record). **This is a new rejection not necessitated by Applicant's amendment.**

Datsenko et al teach the use of an isolated nucleic acid vector comprising λ bet, exo and gam sequences and further comprising a low-copy, temperature sensitive origin of replication. The vector is used in a procedure that 1) has allowed for over 40 different disruptions in the *E. coli* K12 chromosome without failure and 2) has broad use potential, especially in genome analysis of *E. coli* and other bacteria (see entire document, especially the reference to pKD20 in the paragraph bridging pages 6641-6642; page 6640, right column, 2nd full paragraph; and page 6640, last sentence of the Abstract). Datsenko et al further teach that their vectors have specific advantages: 1) optimized ribosome-binding sites for efficient translation of gam; 2) easy elimination at 37°C due to their temperature sensitive origin or replication; and 3) increased efficiency of recombination because the vectors low-copy number prevents competitive inhibition that would inhibit desired recombination events (ibid and page 6644, first full paragraph). Datsenko et al explicitly teach such a nucleic acid in an *E. coli* K12 recombinant host, but note that their method should be "widely useful" and easily extended to use in other bacteria (page 6645, last paragraph).

Datsenko et al do not teach such a vector wherein the λ bet, exo and gam sequences are under the control of a $P_{tac}$ promoter system, including a lacI gene and its native promoter.

Stewart et al teach the use of a $P_{tac}$ promoter system and a lacI gene in methods for promoter recombination in a bacterial host. Stewart et al teach that the $P_{tac}$ promoter is tenfold stronger than lacUV5 (column 26, lines 19-22). Stewart et al also teach the use of a lacI gene when sequences encoding such polypeptides as RecE/T or λ exo or bet are transcribed via lac operon regulatory sequences (column 25, lines 59-67). Stewart et al teach the use of such an isolated nucleic acid in methods of cloning and to promote homologous recombination in *E. coli* (see, e.g., column 11, lines 3-8; column 26, lines 34-35; and columns 45-50, claims 2-11 and 15). Stewart et al teach that "the ability to control the expression of the recombinase sequences such that expression can be regulatable (e.g. inducible) and such that a wide range of expression levels can be achieved is beneficial to the performance of the methods of the invention" (column 24, lines 50-54). Stewart et al further teach an isolated nucleic acid comprising both recombinase (λ exo and bet) and anti-recombinase (λ gam) functions (see reference to pBADαβγ in column 25, lines17-19).

It would have been obvious for one of ordinary skill in the art to combine the $P_{tac}$ promoter of Stewart et al in the vector of Datsenko et al because both Stewart et al and Datsenko et al teach methods of recombinatorial engineering in bacteria which rely upon the expression of λ exo and bet and gam sequences.

One of ordinary skill in the art would have been motivated to combine the vector comprising the λ exo and bet and gam sequences as taught Datsenko et al with the $P_{tac}$ promoter and lacI gene sequences as taught by Stewart et al, because Stewart et al teach that the $P_{tac}$ promoter is a strong promoter and that the use of sequences such as $P_{tac}$ and lacI allow for regulation of the recombinase/anti-recombinase proteins which can be beneficial to the performance of certain methods of recombination, e.g. those taught by Stewart et al (including methods or recombinatorial engineering).

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result when combining the vector of Datsenko et al with the $P_{tac}$ promoter and lacI sequences taught by Stewart et al.

Claims 1-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Datsenko et al (*PNAS* **97**(12)6640-6645, 2000; of record) in view of Stewart et al (US Patent No. 6,355,412; of record), and further in view of Pelletier et al (US Patent No. 6,783,930). **This is a new rejection not necessitated by Applicant's amendment.**

Briefly, as explained above, Datsenko et al in view of Stewart et al teach the use of an isolated nucleic acid vector comprising λ bet, exo and gam sequences under the control of a $P_{tac}$ promoter and lacI. The vector further comprises a low-copy, temperature sensitive origin of replication. The vector is used in procedures that allow for disruptions/recombination in the *E. coli* K12 chromosome without failure and that further are widely useful, for studies of both pathogenic and non-pathogenic bacteria.

Datsenko et al in view of Stewart et al do not teach such a vector wherein the vector is in a *Pseudomonas aeruginosa* or *Mycobacterium tuberculosis* host.

Pelletier et al teach a method for identifying suitable targets for antibacterial agents based on identifying targets of bacteriophage-encoded proteins (see entire document, especially the abstract). Pelletier et al teach that *Pseudomonas aeruginsa* is an exemplary pathogen which can be infected by bacteriophage

and which is a major cause of morbidity and mortality in hospital-based infections (see column 23, lines 56-67 and column 24, lines 1-20). Pelletier et al further teach *Mycobacterium tuberculosis* as an exemplary pathogen which, like *Pseudomonas aeruginosa*, is subject to manipulation with bacteriophage sequences, and which is a human bacterial pathogen (ibid).

It would have been obvious for one of ordinary skill in the art to place the vector as taught by Datsenko et al in view of Stewart et al into hosts such as *P. aeruginosa* and *M. tuberculosis* as taught by Pelletier et al because Datsenko et al and Stewart et al teach that the vector can be used in other bacteria, including other pathogenic bacteria, and Pelletier et al teach that *P. aeruginosa* and *M. tuberculosis* are pathogenic bacteria subject to infection/manipulation by bacteriophage sequences.

One of ordinary skill in the art would have been motivated to place the vector comprising the λ exo and bet and gam sequences as taught Datsenko et al with the $P_{tac}$ promoter and lacI gene sequences as taught by Stewart et al, into the *M. tuberculosis* and *P. aeruginosa* bacteria as taught by Pelletier et al in order to perform genomic manipulations which can lead, either directly or indirectly, to gain knowledge regarding their

genes and ORFS so that these pathogenic bacteria can be inhibited.

Based upon the teachings of the cited references, the high skill of one of ordinary skill in the art, and absent evidence to the contrary, there would have been a reasonable expectation of success to result when placeing the vector of Datsenko et al and Stewart et al into the host organisms taught by Pelletier et al.

The rejection of claims 1, 4 and 7-8 under 35 U.S.C. 103(a) as being unpatentable over Stewart et al (US Patent No. 6,355,412) in view of Poteete et al (*Journal of Bacteriology* **182**(8):2336-2340, 2000) has been WITHDRAWN.

The rejection of claims 1-26 under 35 U.S.C. 103(a) as being unpatentable over Stewart et al (US Patent No. 6,355,412) in view of Poteete et al (*Journal of Bacteriology* **182**(8):2336-2340, 2000), and further in view of Datsenko et al (*PNAS* **97**(12):6640-6645, 2000) has also been WITHDRAWN.

*Response to Arguments*

The response to arguments is rendered moot in view of Examiner's new grounds of rejection under 35 U.S.C. 103(a).

***Conclusion***

No claim is allowed.

Applicant's amendment necessitated the new ground(s) of rejection presented in this Office action. Accordingly, **THIS ACTION IS MADE FINAL.** See MPEP § 706.07(a). Applicant is reminded of the extension of time policy as set forth in 37 CFR 1.136(a).

A shortened statutory period for reply to this final action is set to expire THREE MONTHS from the mailing date of this action. In the event a first reply is filed within TWO MONTHS of the mailing date of this final action and the advisory action is not mailed until after the end of the THREE-MONTH shortened statutory period, then the shortened statutory period will expire on the date the advisory action is mailed, and any extension fee pursuant to 37 CFR 1.136(a) will be calculated from the mailing date of the advisory action. In no event, however, will the statutory period for reply expire later than SIX MONTHS from the date of this final action.

Certain papers related to this application may be submitted to the Art Unit 1636 by facsimile transmission. The faxing of such papers must conform with the notices published in the Official Gazette, 1156 OG 61 (November 16, 1993) and 1157 OG 94 (December 28, 1993) (see 37 C.F.R. § 1.6(d)). The official fax telephone number for the Group is (571) 273-8300. Note: If

Applicant *does* submit a paper by fax, the original signed copy should be retained by Applicant or Applicant's representative. NO DUPLICATE COPIES SHOULD BE SUBMITTED so as to avoid the processing of duplicate papers in the Office.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to (571) 272-0547.

Patent applicants with problems or questions regarding electronic images that can be viewed in the Patent Application Information Retrieval system (PAIR) can now contact the USPTO's Patent Electronic Business Center (Patent EBC) for assistance. Representatives are available to answer your questions daily from 6 am to midnight (EST). The toll free number is (866) 217-9197. When calling please have your application serial or patent number, the type of document you are having an image problem with, the number of pages and the specific nature of the problem. The Patent Electronic Business Center will notify applicants of the resolution of the problem within 5-7 business days. Applicants can also check PAIR to confirm that the problem has been corrected. The USPTO's Patent Electronic Business Center is a complete service center supporting all patent business on the Internet. The USPTO's PAIR system provides Internet-based access to patent applications to view the scanned images of their own application file folder(s) as well as general patent information available to the public.

For all other customer support, please call the USPTO Call Center (UCC) at (800) 786-9199.

Any inquiry concerning rejections or objections in this communication or earlier communications from the examiner should be directed to Walter Schlapkohl whose telephone number is (571)

272-4439. The examiner can normally be reached on Monday through Thursday from 8:30 AM to 6:00 PM. The examiner can also be reached on alternate Fridays.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Dr. Remy Yucel can be reached at (571) 272-0781.

Walter A. Schlapkohl, Ph.D.
Patent Examiner
Art Unit 1636

September 25, 2006

NANCY VOGEL
PRIMARY EXAMINER